SCIENTIFIC LETTER

Dark chocolate improves endothelial and platelet function

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The effects of chocolate on cardiovascular health are still a matter of debate. Chocolate may adversely affect cardiovascular risk because of its effects on glucose, lipids, and body weight or potentially favour cardiovascular health through antioxidative effects of chocolate ingredients, such as flavonoids (present in dark but not white chocolate).

Endothelial dysfunction and platelet activation are cornerstones in the pathogenesis of atherothrombosis, leading to vasoconstriction, thrombus formation, and inflammation. Smoking is a major cardiovascular risk factor. The mechanisms promoting atherothrombosis in smokers primarily include increased oxidative stress that enhances proatherogenic processes such as low density lipoprotein oxidation and inactivation of endothelium derived nitric oxide. Platelets contribute both to acute coronary syndromes and to the progression of atherothrombosis. Both active and passive cigarette smoking has consistently been shown to induce endothelial dysfunction. Therefore, smokers serve as an ideal model to study the beneficial vascular effects of antioxidant strategies such as dark chocolate.[1]

The goal of the present study was to investigate whether the beneficial antioxidant effect of polyphenol-rich dark chocolate can induce an improvement of endothelial and platelet function in healthy volunteers with known endothelial dysfunction and platelet hyperreactivity.

METHODS

Twenty five male smokers were enrolled in the study after giving written informed consent. Women were excluded for known sex hormone induced differences in vascular tone and reactivity. All study participants did not take any medication, including vitamins or dietary supplements. The local institutional ethical review board approved the protocol.

To assess the effect of dark chocolate on endothelial function, five chronic smokers underwent a preliminary protocol. The subjects were studied by high resolution ultrasound before (baseline measurements) and after ingestion of 40 g of dark chocolate (74% cocoa, Nestlé Noir Intense; Nestlé SA, Vevey, Switzerland) and a 24 hour period of abstinence from polyphenol-rich food (such as black or green tea, onions, apples, cabbage, wine, and cocoa products). Endothelial function was reassessed by ultrasonography at two, four, eight, and 24 hours after chocolate ingestion.

Given the positive results of the preliminary study, 20 subjects were randomly divided into two parallel groups. Endothelial function and shear stress dependent platelet function were assessed at baseline after 24 hours' abstinence from food rich in polyphenols and two hours after ingestion of chocolate, either 40 g of dark chocolate or 40 g of white chocolate (4% cocoa, Nestlé Galak). In both parts of the study, subjects were studied after a fasting period of eight hours and a smoke-free interval of at least 30 minutes (mean 80 minutes) before each experiment.

Flow mediated dilatation (FMD) was measured by highly sensitive ultrasonography of the brachial artery.[2]

For measurement of shear stress dependent platelet function, citrated whole blood (200 µl) was circulated in polystyrene wells at a shear rate of 1875/s for two minutes with a rotating Teflon cone.[3] Wells were washed, stained with May-Grünwald, and analysed with a microscope connected to a computed image analysis system (ImageJ 1.31v; National Institutes of Health, Bethesda, Maryland, USA). Results are expressed as the percentage surface covered by platelets. Antioxidant status was measured with the total antioxidant status kit (Randox Laboratories, Crumlin, UK).

Treatment effects and differences between groups were evaluated by paired or unpaired *t* test and analysis of variance for repeated measures, respectively (JMP5.0.1; SAS Institute, Cary, North Carolina, USA). All probability values are two tailed, and a probability value of $p < 0.05$ was considered significant. Results are presented as mean (SEM).

RESULTS

Baseline characteristics did not differ between the groups. No change in glucose and lipids were detected two hours after chocolate ingestion.

Dark chocolate significantly improved FMD after two hours compared with baseline (7.0 (0.7)% *v* 4.4 (0.9)%, $p = 0.026$) (fig 1A). The effect of dark chocolate on FMD lasted about eight hours (fig 1B). White chocolate had no effect on FMD. Baseline arterial diameters in the white and dark chocolate groups did not differ and diameter did not change over time within groups. Vascular flow response did not change in either groups (data not shown). Glyceryl trinitrate induced vasodilatation was not affected by either dark or white chocolate (dark chocolate, 9.0 (1.6)% *v* 7.3 (1.0)%, $p = 0.16$, and white chocolate, 8.7 (0.8)% *v* 8.4 (1.0), $p = 0.43$).

Two hours after dark chocolate ingestion shear stress dependent platelet function was reduced from 5.0 (0.6)% to 3.2 (0.4)% ($p = 0.03$ versus baseline) (fig 1C). No significant effect was seen in the white chocolate group.

Total antioxidant status significantly increased two hours after ingestion of dark chocolate but not after ingestion of white chocolate (dark chocolate, 1.22 (0.02) *v* 1.25 (0.02), $p = 0.03$, and white chocolate, 1.25 (0.04) *v* 1.24 (0.03), not significant).

DISCUSSION

Dark but not white chocolate induced a rapid and significant improvement of endothelial and platelet function in healthy smokers 2–8 hours after ingestion. Cigarette smokers exhibit increased atherogenic potential, as they consistently have endothelial and platelet dysfunction, which are associated with an increased cardiovascular risk.[1]

Improving the interplay between platelets and the vascular endothelium may beneficially affect cardiovascular disease. The observed improvement in FMD indicates a specific effect of dark chocolate on the endothelium, as endothelium independent vasodilatation to glyceryl trinitrate was not influenced. These findings complement several recent

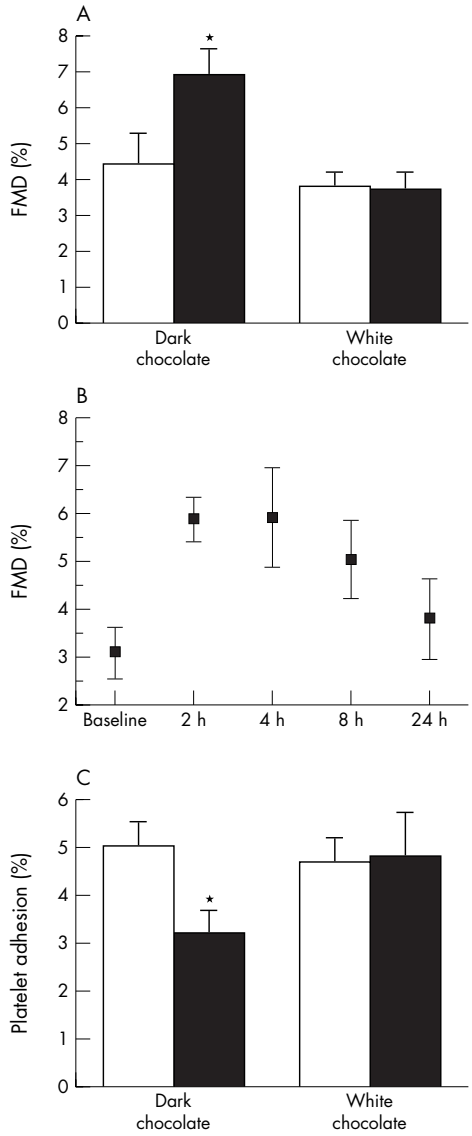

Figure 1 (A) Flow mediated dilatation (FMD) significantly improved two hours after ingestion of 40 g of dark chocolate, indicating improved endothelial function. White chocolate had no effect on FMD. *p = 0.026 between baseline (white bars) and two hours after ingestion of chocolate (black bars). n = 10 in each group. (B) The increase in FMD after ingestion of dark chocolate lasted for eight hours. p = 0.038 (analysis of variance). (C) Shear stress dependent platelet adhesion was significantly reduced by dark but not by white chocolate. *p = 0.03 between baseline (white bars) and two hours after ingestion of chocolate (black bars).

observations of the nitric oxide dependent vasodilatative properties of flavonol-rich cocoa products in healthy subjects and in elderly patients with isolated systolic hypertension.[4]

In the present study, platelet adhesion under high shear stress conditions (typically occurring at the site of severely stenotic or disrupted plaques) was significantly reduced two hours after dark chocolate ingestion. The high flavonoid content of dark chocolate may potentially explain the mechanisms for the reduced platelet activation. Besides direct antioxidant capacities, flavonoids may influence 5-lipoxygenase activity and alter signal transduction pathways through antioxidant independent mechanisms. Indeed, endothelial dysfunction and platelet activation are partially caused by inactivation of endothelium derived nitric oxide by reactive oxygen species. Dark chocolate has a much higher polyphenol (a heterogeneous group of antioxidants) content per gram than do other antioxidant-rich foods such as wine, tea, or berries. Therefore, only a small daily treat of dark chocolate may substantially increase the amount of antioxidant intake and beneficially effect vascular health. Further studies are needed to elucidate the active compounds and the long term effects of polyphenols on health.

In conclusion, dark chocolate exerts favourable effects on endothelial function and platelet aggregation. These findings are most likely mediated by the antioxidant effect of dark chocolate.

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